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A new species of *Andricus* Hartig gallwasps from California (Hymenoptera: Cynipidae: Cynipini) galling *Notholithocarpus* (Fagaceae)

James A. Nicholls, George Melika, John D. Demartini & Graham N. Stone

Abstract

Andricus notholithocarpi, **n. sp.** (Hymenoptera, Cynipidae, Cynipini) from California, USA, that induces galls on *Notholithocarpus* (Fagaceae) is described. Description, diagnoses, biology, and host associations for the new species are given. The different gall types induced by this species are described in detail. The new taxon is supported by morphological and molecular data.

Keywords: Cynipidae, *Andricus notholithocarpi*, *Notholithocarpus*, biology, morphology

Zusammenfassung

Beschreibung von *Andricus notholithocarpi*, **n. sp.** (Hymenoptera, Cynipidae, Cynipini) aus California, USA, eine Gallenwespe an *Notholithocarpus* (Fagaceae). Beschreibung, Diagnose und Lebensweise für die neue Art werden geben. Die verschiedenen Galltypen, welche diese Art induziert werden im Detail beschrieben. Der Artstatus wird sowohl von morphologischen als auch molekularen Daten unterstützt.

Contents

1 Introduction 17
2 Material and Methods 18
3 Results 21
4 Discussion 23
5 References 23

1 Introduction

Oak gallwasps (Hymenoptera: Cynipidae: Cynipini) are by far the most species-rich group of gallwasps, with about 1,000 known species in 41 genera worldwide (Melika & Abrahamson 2002; Csóka et al. 2005; Ronquist et al. 2015), of which about 680 species are known from the Nearctic (Burks 1979; Melika & Abrahamson 2002 and authors' unpublished data). The oak gallwasp tribe Cynipini is associated with both *Quercus* L. and non-*Quercus* genera of Fagaceae, including *Castanea* Miller, *Castanopsis* (D. Don), and *Lithocarpus* Blume in the Eastern Palaearctic, and *Chrysolepis* Hjelmq. and *Notholithocarpus* Manos, Cannon & S.H.Oh in North America (Csóka, Stone & Melika 2005; Govaerts & Frodin 1998; Manos et al. 2001, 2008; Melika 2006; Tang et al. 2011, 2016).

The California Floristic Province of western North America is rich in palaeo-endemic.

Plant taxa, including two Fagaceae genera: the monotypic *Notholithocarpus* containing the tanbark oak, *Notholithocarpus densiflorus*, and the chinquapin oak genus *Chrysolepis*, containing two species *Chrysolepis chrysophylla* (Douglas ex Hook.) Hjelmq. and *Ch. sempervirens* (Kell.) Hjelmq. Recent phylogenetic analysis has shown *Chrysolepis* to be a strongly supported sister group to the large genus *Lithocarpus* (Manos et al. 2008), which is represented by 325 species in Asia and Malesia (Govaerts & Frodin 1998). Only two described cynipid species are known to associate with *Lithocarpus*: *Cycloneuroterus formosanus* Tang & Melika from Taiwan (Tang et al. 2011), and *Neuroterus haasi* Kieffer, from India (Dalla Torre & Kieffer 1910). The genus *Chrysolepis* is also only galled by a small number of cynipid species, including the described species *Dryocosmus castanopsidis* (Beutenmueller) and *D. rileypokei* Morita & Buffington (Buffington & Morita 2009), and an undescribed leaf galler (Weld 1957a).

In contrast, although North American *Notholithocarpus densiflorus* was originally classified within *Lithocarpus* on the basis of morphology, it is now seen as distant from *Lithocarpus* and placed within the clade containing *Quercus*, *Castanea* and *Castanopsis* on the basis of DNA sequence data (Manos et al. 2008; Oh & Manos 2008). The only cynipid species known from *Notholithocarpus densiflorus*, from California, is *Andricus mendocinensis* Weld, which induces subterranean root galls and is known only from asexual generation ♀♀ (Weld 1957b).

Here we describe a new species, *Andricus notholithocarpi* Melika, Nicholls & Stone, **n. sp.** known only from an asexual generation, which induces galls on *Notholithocarpus densiflorus* in California.

Acknowledgements

We are grateful to Szabina Schwéger of the Plant Health and Molecular Biology Laboratory, National Food Chain Safety Office, Budapest, Hungary, who took the pictures of the species.

2 Materials and methods

Specimen collection: Trees of *Notholithocarpus densiflorus* were searched for galls to photograph and to collect at three localities in Humboldt County, California, USA. A site 20km E of Arcata (40.93° N, 123.86° W) was visited on 01.IV.2008, 08.III.2009, 24.VIII.2009, 29.IX.2009, 25.X.2009 and 26.XII.2009; two sites north of Orick (41.39° N, 124.03° W) were visited on 21.IX.2008 and 18.I.2009; and a site along Bald Hills Road, 40km NE of Arcata (41.16° N, 123.81° W) was visited on 04.IV.2009 and 20-21.VI.2009. All parts of the trees were searched, with particular focus on new growth. Four different gall morphologies were discovered. All galls found were reared in plastic vials with mesh lids at ambient conditions. A small proportion of each gall type were dissected immediately after collection and any larvae found were stored in 100% ethanol.

Molecular analysis: Whole genomic DNA was extracted from 1–3 individuals of each gall type using either small sections of dissected larvae or a single leg of reared adults following the chelex/Proteinase K protocol described in Nicholls et al. (2010). Sequence data were obtained for sections of three genes previously shown to be informative for delimiting cynipid species (see Nicholls et al. 2012): the mitochondrial cytochrome *b* gene (cytb), and the nuclear genes long-wavelength opsin (opsin) and the second ribosomal internal transcribed spacer region (ITS2). PCR products were cleaned up using a standard SAP/ExoI protocol, then sequenced in both directions using BigDye v3.1 terminator chemistry and run on an ABI3730 capillary sequencer. Base calls were confirmed by eye using Sequencher version 4.10.1 (Gene Codes Corporation 1995). The number of base differences for each gene between all individuals were determined in PAUP* v4.0b10 (Swofford 1998).

Morphological descriptions: The terminology used to describe gallwasp morphology follows other recent cynipid studies (Melika 2006; Melika et al. 2010; Liljeblad et al. 2008). Abbreviations for forewing venation follow Ronquist & Nordlander (1989), and cuticular surface terminology follows Harris (1979). Measurements and abbreviations used here include: F1–F12 for the 1st and subsequent flagellomeres; POL (post-ocellar distance) for the distance between the inner margins of the posterior ocelli; OOL (ocellar-ocular distance) for the

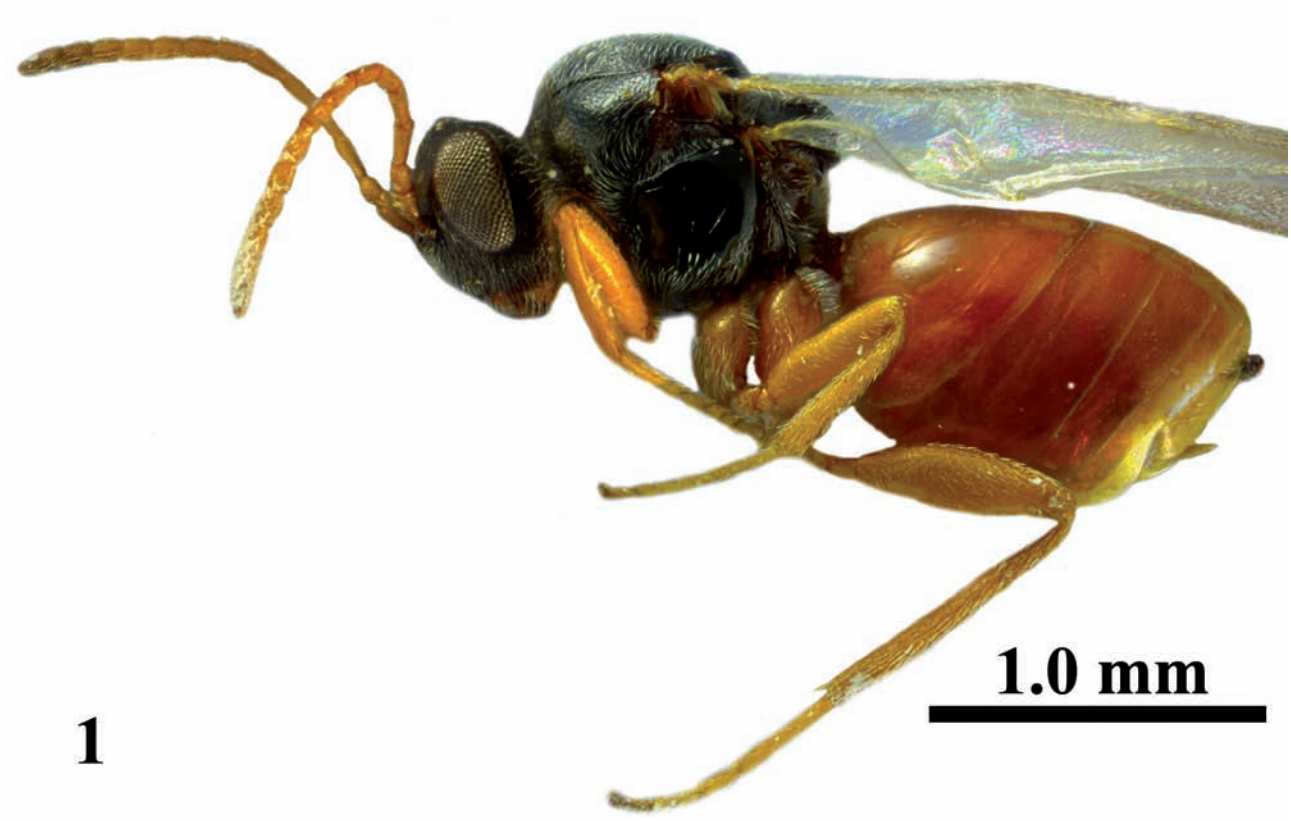
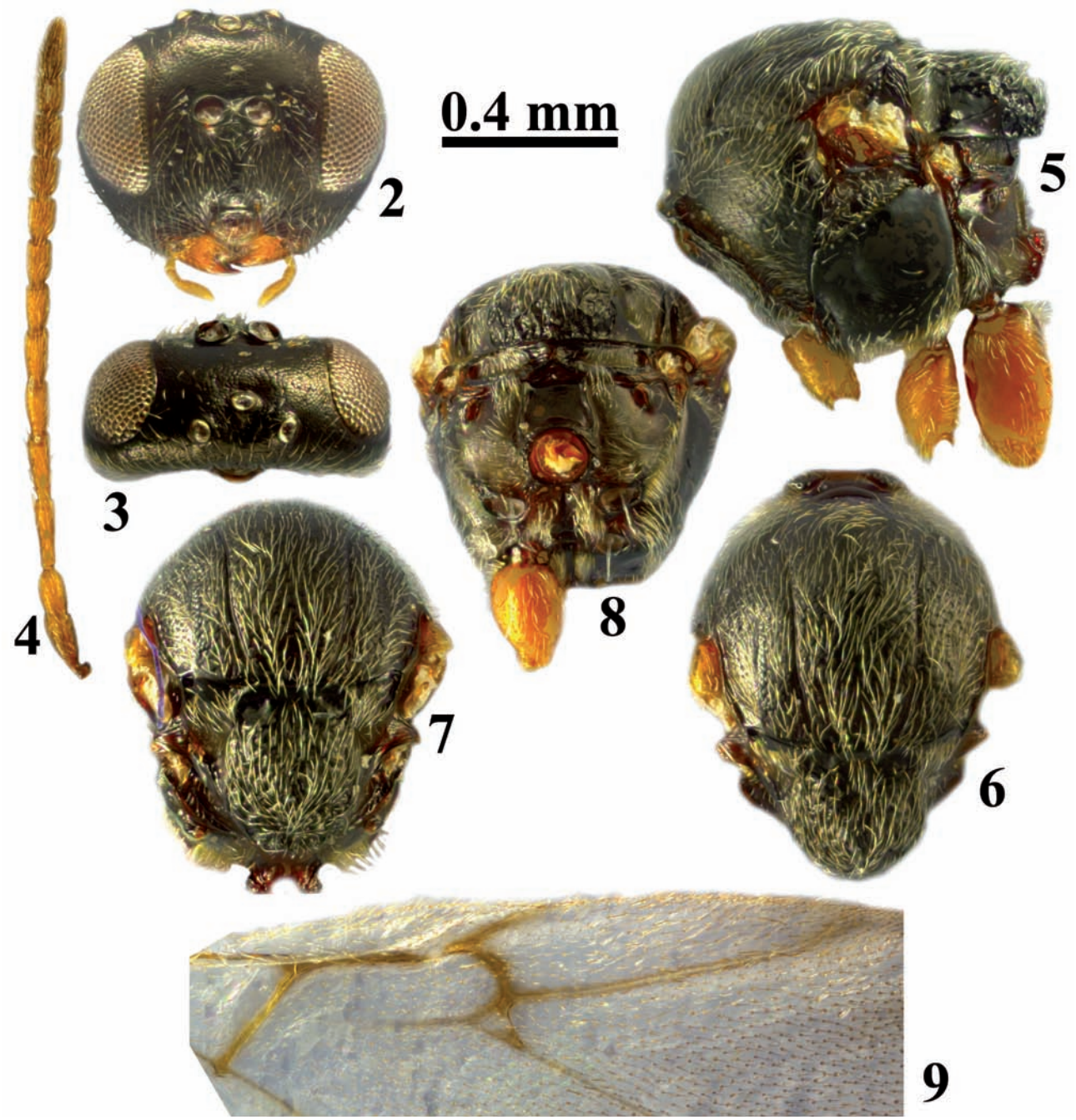


Fig. 1. *Andricus notholithocarpi*, n. sp., ♀, general view. – Scale: 1 mm.

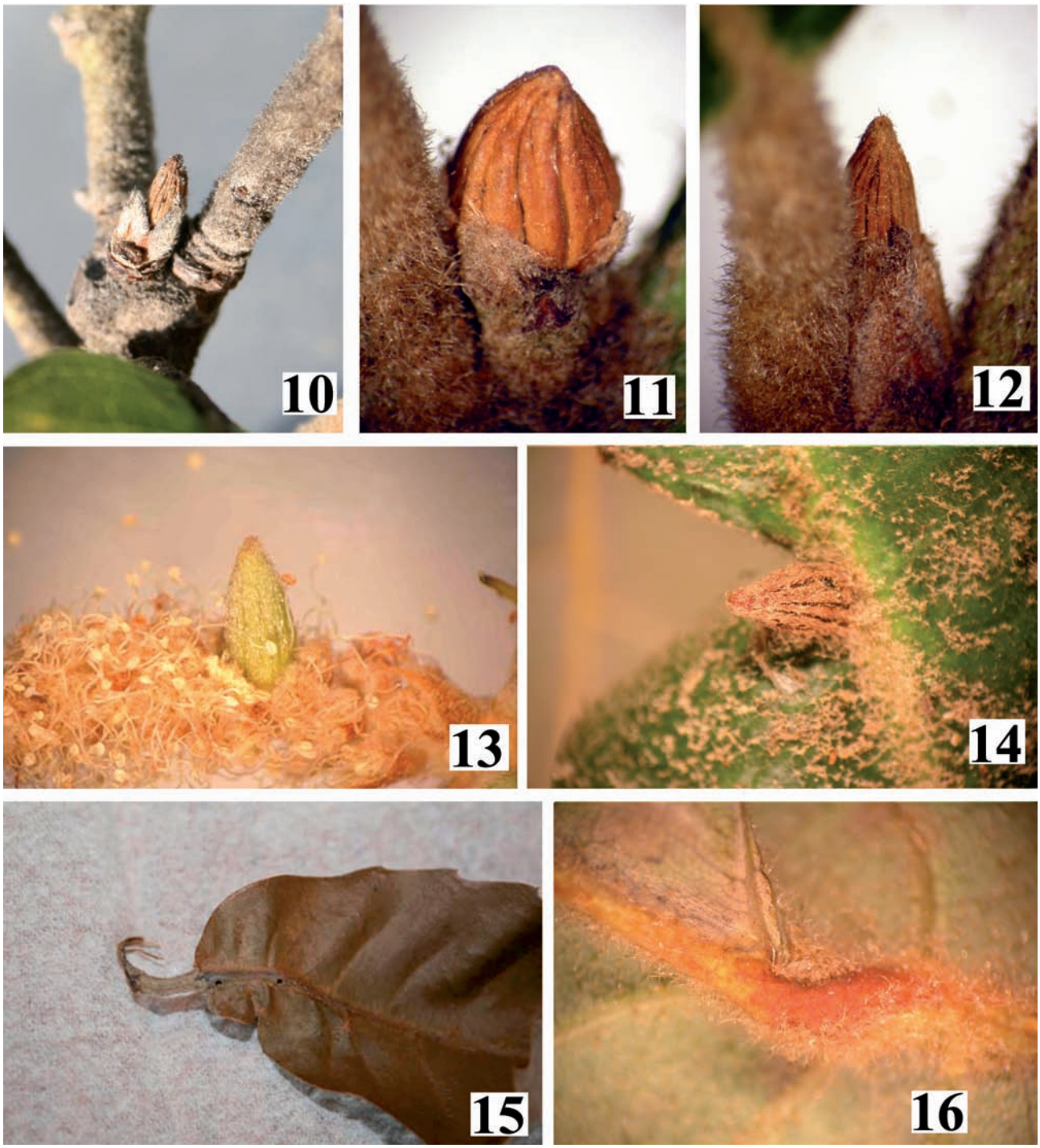


Figs. 2–9. *Andricus notholithocarpi*, n. sp., ♀ – 2–3, head. 4, antenna. 5, mesosoma, lateral view. 6, mesoscutum and mesoscutellum, dorsal view. 7. mesosoma, dorsal view. 8, metascutellum and propodeum, posterodorsal view. 9, forewing, part. – Scale: 0.4 mm.

distance from the outer edge of a posterior ocellus to the inner margin of the compound eye; and LOL (lateral-frontal ocelli distance) for the distance between lateral and frontal ocelli. The width of the forewing radial cell is measured from the margin of the wing to the Rs vein.

Bright field images of adults were produced with a digital Leica DC500 camera attached to a Leica DM2700M compound microscope using the LAS Store&Recall software, followed by processing in Adobe Photoshop 6.0. Gall images were taken in the field by J.D. DeMartini and J.A. Nicholls.

The type material is deposited in the Plant Health and Molecular Biology Laboratory, National Food Chain Safety Office, Budapest, Hungary.



Figs. 10–16. *Andricus notholithocarpi*, n. sp., galltypes – 10–12, galls on buds (spCAb4). 13, galls on catkins (spCAc7). 14, galls on leaves (spCAl15). 15–16, galls in swollen leaf midribs (spCAl8).

3 Results

Andricus notholithocarpi Melika, Nicholls & Stone, **n. sp.**

Type material: Holotype ♀: „USA, California, Prairie Creek Redwood State Park, leg. J. Nicholls, 2009.01.18 (CA1294), leaf gall spCAl15_1, ex *Notholithocarpus densiflorus*"; 1 ♀paratype with the same label as the holotype.

Etymology: Named after the host plant, *Notholithocarpus*.

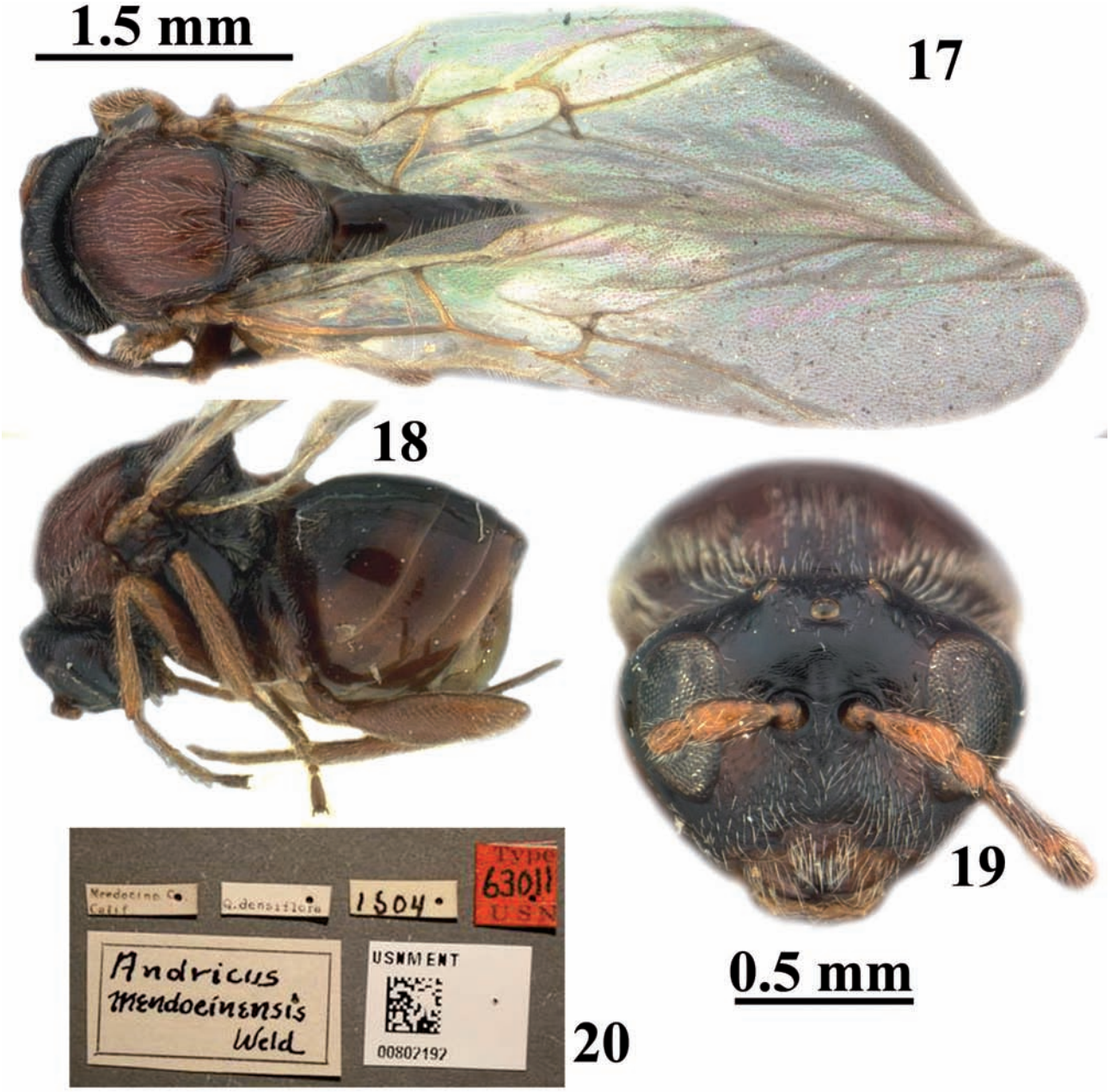
Diagnosis: Tarsal claws with a distinct basal lobe, the gena slightly broadened behind the eye, so it falls into asexual *Andricus* Hartig *sensu* WELD (1952). The only other cynipid species known to associate with *Notholithocarpus densiflorus* is *Andricus mendocinensis* Weld (Figs. 17–20), also known only from California and inducing subterranean root galls (WELD 1957b). Morphologically the new species closely resembles *A. mendocinensis*. In *A. notholithocarpi*, **n. sp.** (Figs. 1–16) the mesosoma is entirely black, metasoma reddish brown, the head is rounded in frontal view, the gena slightly broadened behind the eye, POL 1.6x as long as OOL, the frons and interocellar area alutaceous, scutellar foveae rounded, divided by a broad central median carina, the prominent part of the ventral spine of the hypopygium only 3.0x longer than broad in ventral view; while in *A. mendocinensis* the mesosoma and metasoma are uniformly dark brown, the head is triagular in frontal view, narrowing towards the ventral edge, the gena strongly broadened behind the eye, POL1.3x as long as OOL, the frons and interocellar area smooth, glabrous, scutellar foveae transverse, divided by a narrow central median carina, the metasoma as long as the head+mesosoma, the prominent part of the ventral spine of the hypopygium at least 6.5x longer than broad in ventral view.

Description: Asexual ♀ (holotype) (Figs. 1–16). Head and mesosoma dark brown to black, clypeus, mandibles, mouthparts, antennae, legs and metasoma uniformly reddish brown.

Head alutaceous, with dense white setae, more dense on lower face, 2.1x broader than long from dorsal view; 1.3x broader than high in frontal view and slightly broader than mesosoma. Gena alutaceous, slightly broadened behind eye, 2.0x narrower than cross diameter of eye. Malar space alutaceous, without striae; height of eye 2.1x longer than length of malar space. Inner sides of compound eyes parallel. POL1.3x as long as OOL; OOL 1.5x longer than diameter of lateral ocellus, slightly longer than LOL; ocelli ovate, all three equal in size. Transfacial distance 1.2x longer than height of eye and 1.4x longer than height of lower face (distance between antennal rim and ventral margin of clypeus); diameter of antennal torulus 2.1x longer than distance between them, distance between torulus and eye margin 1.5x as long as diameter of torulus. Lower face alutaceous, without striae, with elevated median area, with dense setae. Clypeus rectangular, flat, broader than high, alutaceous, with deep anterior tentorial pits, distinct epistomal sulcus and clypeo-pleurostomal line; ventrally rounded, emarginate, not incised medially. Frons and interocellar area smooth, glabrous, vertex, occiput uniformly alutaceous. Postgena smooth, glabrous, postocciput around occipital foramen impressed, smooth, glabrous, with parallel striae; posterior tentorial pits large, deep, elongate; postgenal bridge narrow, 2.3x higher than broad; occipital foramen higher than height of postgenal bridge. Antenna with 11 flagellomeres, longer than head+mesosoma; pedicel 2.7x as long as broad, F1 1.6x shorter than length of scape+pedicel, 1.2x as long as F2, F2 slightly longer than F3, F3 slightly longer than F4, F5 longer than F6, subsequent flagellomeres nearly equal in length, F11 1.9x longer than F10; placodeal sensilla on F3–F11, in numerous rows.

Mesosoma longer than high in lateral view. Pronotum alutaceous, with white setae, without striae laterally, emarginate along lateral edge. Mesoscutum alutaceous with dense white setae; nearly as long as broad (width measured across base of tegulae); notauli complete, uniformly impressed along full length; median mesoscutal line absent; anterior parallel lines invisible, parapsidal lines not impressed, indicated by more dull sculpture than rest of mesoscutum, reaching half the length of mesoscutum; parascutal carina broad, deep, anteriorly reaching anterior edge of tegula. Transscutal articulation deep, distinct. Mesoscutellum slightly longer than broad, with subparallel sides laterally; shorter than mesoscutum, uniformly rugose, overhanging metanotum; scutellar foveae rounded, with smooth, glabrous bottom, divided by broad central elevated carina. Mesopleuron, including speculum, smooth, glabrous, without setae, with few white setae on ventral edge; mesopleural triangle coriaceous, with dense white setae and some delicate irregular short wrinkles. Metapleural sulcus reaching mesopleuron at slightly above half its height; dorsal and lateral axillar areas alutaceous, with dense setae; axillar carina broad, with longitudinal striae; subaxillular bar narrow, smooth, glabrous, at the most posterior end slightly shorter than the height of metanotal trough. Metascutellum delicately coriaceous, as high as the height of smooth, glabrous ventral impressed area; metanotal trough smooth, glabrous, with dense white setae. Lateral propodeal carinae distinct, slightly bent outwards in posterior third, central propodeal area smooth, glabrous, without wrinkles or setae; lateral propodeal area smooth, with dense white setae; nucha short, with delicate longitudinal sulci dorsolaterally and laterally.

Forewing slightly longer than body, with pale brown veins, margin with cilia; radial cell 2.4x longer than broad, R1 and Rs nearly reaching wing margin; areolet triangular, well-delimited by distinct veins; Rs+M projection reaching basalis at mid-height. Tarsal claws with a basal lobe.



Figs. 17–20. *Andricus mendocinensis,* ♀ – 17–18, general habitus. 19, head, frontal view. 20, type labels. – Scales: Figs. 17–18: 1.5 mm; Fig. 19: 0.5 mm. Photos from the National Museum of Natural History online types collection.

Metasoma longer than head+mesosoma, longer than high in lateral view, smooth, glabrous, without setae; the prominent part of the ventral spine of the hypopygium only 3.0x longer than broad in ventral view, with few white setae, ventrally. Body length 2.8–2.9 mm (n = 2).

Molecular results. DNA sequence data were obtained for each of the three genes from at least one individual of gall inducer from each of the four observed gall types. Sequence data for all three genes were obtained from the holotype individual. In total, five cytb sequences were obtained, with only two bases out of a total sequence length of 433 base pairs differing (0.46% variation). Identical opsin sequences were obtained from five individuals. Seven individuals were sequenced for ITS2; two different allelic sequences were found that differed by only a single base. These very low levels of variation confirm that

the four gall types are all induced by *A. notholithocarpi*, **n. sp**. Sequences are deposited in GenBank, accessions MF353455-MF353488.

Phylocomments: Preliminary analyses of cytb sequence data suggest this species falls within the same major cynipid clade as the North American species *Andricus quercuspetiolicola* (Bassett), *A. quercusfrondosus* (Bassett), *A. cinnamomeus* Ashmead, *A. quercusfoliatus* (Ashmead), *A. reticulatus* Bassett, *Callirhytis clavula* (Osten Sacken); *Andricus wiltzae* Fullaway and *A. opertus* (Weld), and more distantly *Andricus quercusutriculus* (Bassett) and *A. foliaformis* Gillette.

Gall: Four different gall types induced by this species were found (Figs. 10–16). Three are very similar, differing only by the plant organ on which they occur. The fourth probably represents an early developmental stage of the typical gall induced on a leaf; perhaps with development stopped due to parasitism.

1. Galls in buds (authors' field code spCAb4) (Figs. 10–12) – small (~3–5 mm long) pip gall in an axillary bud, with longitudinal ridges along the gall surface. Pale green when developing, rusty-brown when mature. Collected in October, December, April and May.

2. Galls on catkins (authors' field code spCAc7) (Fig. 13) – small conical gall on staminate catkins, with delicate longitudinal ridges along gall, several mm long, pale green when developing. Collected in August, September and December. The gall is very similar to spCAb4 and spCAl15, just on a different plant organ.

3. Galls on leaves (authors' field code spCAl15) (Fig. 14) – small conical leaf gall, on edge of leaf, causing infolding of leaf edge and bending of midrib, small longitudinal ridges on outside of gall. Collected in June, August, October, December, January and March. The gall is very similar to spCAc7 and spCAb4, just on a different plant organ.

4. Galls in swollen leaf midribs (authors' field code spCAl8) (Figs. 15–16) – swollen leaf midrib or petiole, green or yellowish-green; usually causes midrib of leaf to bend. Collected in June, September, October, January and April. No adult gall-inducers were reared from this gall type; only unidentified cynipid inquilines (Synergini) and parasitoids from the superfamily Chalcidoidea were reared. However, remains of larval gall inducers were dissected from this gall type.

Biology: Galls start to develop in June-August at the same time as new leaf and flower development. Gall and larval development continues during September-December, larvae overwinter in galls and adults emerge in early spring of the following year. Currently known only from *Notholithocarpus densiflorus*.

Distribution: Currently known only from California, USA.

4 Discussion

Cynipini host-plant associations are extremely conserved phylogenetically (Stone et al. 2009), both across different subgenera of oaks and across other non-oak Fagaceae (*Castanea*, *Castanopsis*, *Lithocarpus*, and *Notholithocarpus*) (Tang et al. 2011, 2016). The morphological similarity of the two species *A. mendocinensis* and *A. notholithocarpi*, **n. sp.**, associated with *Notholithocarpus* suggests they represent a monophyletic lineage. No other species have yet been found that induce galls on *Notholithocarpus*; however, if others are found, we would expect them to be related to the pair of known *Notholithocarpus*-associated species. The apparent affinities of the *Notholithocarpus*-associated *Andricus* with Nearctic *Andricus* attacking *Quercus* species suggests that they are the result of a relatively recent host plant shift to *Notholithocarpus* from *Quercus*, and do not represent a deeply divergent lineage in the Cynipini gallwasp phylogeny. However, further sampling and phylogenetic analyses are required to confirm this.

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Authors' addresses:

Dr. James A. Nicholls, Institute of Evolutionary Biology, University of Edinburgh, King's Buildings, Edinburgh EH9 3FL, United Kingdom;
e-mail: james.nicholls@ed.ac.uk

Dr. George Melika, Plant Health and Molecular Biology Laboratory, National Food Chain Safety Office, Directorate of Plant Protection, Soil Conservation and Agri-environment, Budaörsi str. 141-145, Budapest 1118, Hungary;
e-mail: melikageorge@gmail.com Corresponding author

Dr. John D. Demartini, Department of Biological Sciences, Humboldt State University, 1 Harpst St., Arcata, CA 95521, USA;
e-mail: jdd2@humboldt.edu

Dr. Graham N. Stone, Institute of Evolutionary Biology, University of Edinburgh, King's Buildings, Edinburgh EH9 3FL, United Kingdom;
e-mail: graham.stone@ed.ac.uk

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